

THE ACTIVITY OF THE CONTRACTILE VACUOLE IN A SUCTORIAN (TOKOPHRYA INFUSIONUM)¹

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This paper deals with a study of the behavior of the contractile vacuole during several functional phases in the life of *Tokophrya infusionum*, a protozoon of the Class Suctorina.

The *Tokophrya* has proved to be exceptionally favorable: (a) The sedentary habit of the adult facilitates observation on the contractile vacuole during all periods of its activity. (b) The food supply can be regulated since the organism feeds only on living ciliates. (c) The contents of the prey are incorporated by being sucked out through the predatory tentacles of the host. This eliminates the complication of having water introduced, a usual accompaniment of the formation of food vacuoles in other protozoa. (d) During reproduction a parent organism persists, since *Tokophrya* reproduces by the formation of a succession of "embryos" in temporary brood pouches.

GENERAL DESCRIPTION OF TOKOPHRYA

Tokophrya, a fresh water, sessile suctorian, possesses a spheroidal or pyriform body with an average diameter of 17 to 50 micra. Two bundles of straight, motionless, sucking tentacles (5-20 in each bundle) extend, one from each side of the body (Fig. 1A). The contractile vacuole, of which there may be two, rarely three, has an average maximum diameter of 10 micra. Feeding starts as soon as the prey, generally consisting of small living ciliates, becomes attached to one or several tips of the extended tentacles. One *Tokophrya* may feed simultaneously on ten or more ciliates, each of which may be several times the size of the *Tokophrya*. In a starved *Tokophrya* the body is translucent and the macronucleus is then clearly visible. During feeding, the body becomes opaque and filled with numerous dark food vacuoles. With time these vacuoles become increasingly translucent and finally disappear within 24-48 hours. The capture of the swimming prey depends upon its chance contact with the tentacles of the *Tokophrya*. Shortly after being caught, the prey becomes motionless and, a few seconds later, granules of cytoplasm from the prey can be seen to flow through the hollow tentacles into the host. Within 5 to 10 minutes the prey progressively shrinks in size and, after 25 minutes or more, only a spherical remnant is left, which becomes detached and disintegrates. It is of interest to note that the contractile vacuole of the prey continues to pulsate until just before the prey is reduced to the discarded small remnant.

Tokophrya reproduces by endogenous budding, the bud being produced within a temporary vacuole, the so-called "brood pouch." A well-fed individual is able

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to produce as many as twelve embryos successively during 24 hours. The first indication of budding is the formation of the brood pouch which appears as a large triangular vacuole at the apical region of the body. After a few minutes the vacuole becomes crescentic as it progressively encircles one of the two mitotically produced

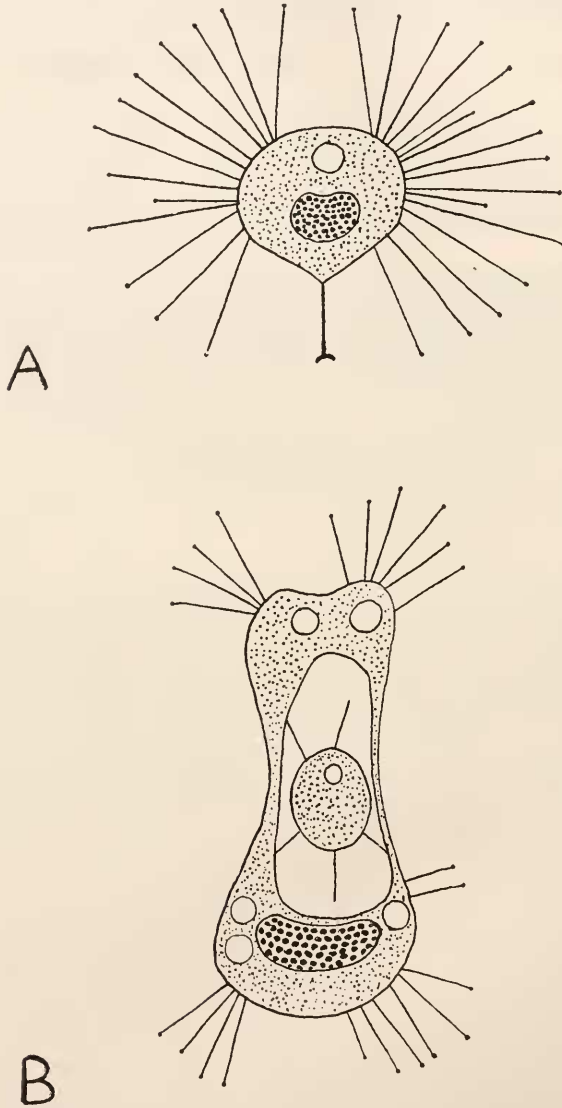


FIGURE 1. A. An adult *Tokophrya infusionum* with two bunches of tentacles, a stalk with an attachment disc, one contractile vacuole and a macronucleus. B. An abnormal case of a *Tokophrya infusionum* in which a young adult persists in the brood pouch. There are five bunches of short tentacles and five contractile vacuoles. The enclosed young *Tokophrya* has one contractile vacuole and five tentacles, the tips of three of which are in contact with the wall of the brood pouch.

miconuclei, together with some cytoplasm and a small portion of the macronucleus. The embryo, thus constituted, is pinched off, becomes ciliated and rotates within the brood pouch for about 10 to 20 minutes before it is ejected to the exterior. The whole process, from the time of the formation of the brood pouch until the liberation of the embryo, lasts for a little more than an hour. The liberated embryo swims for several minutes to several hours after which it becomes attached, loses its cilia and is transformed in the next few minutes into a mature form. The development and subsequent ejection of the embryo in Suctorina has been fully described by Collin (1911, 1912), Penard (1917-23), Pestel (1931), Noble (1932).

MATERIAL AND METHODS

The material for this study was obtained in the spring of 1948 through the courtesy of Dr. Daniel Lilly, St. John's College, Brooklyn, New York, from a pond in the vicinity of Laurelton, New York. From one of several mass cultures, 20 pure line cultures of *Tokophrya infusionum* were started. These were maintained in standing drops on microscopic slides bounded by walls of thick vaseline or a mixture of paraffin and beeswax. All the cultures were kept in large moist chambers and transfers were made weekly.

Bacteria-free cultures were obtained by transferring a free swimming embryo through several washes of autoclaved Great Bear Spring water. The ensuing progeny were maintained throughout in autoclaved water. The food supply consisted of bacteria-free cultures of *Tetrahymena geleii*. These were grown in a proteose-peptone medium. The cultures of *Tokophrya* were fed with *Tetrahymena* three times a week and were transferred to fresh media every three to four weeks.

The contractile vacuole always undergoes complete contraction. Its pulsation rate was recorded by means of a stop watch and its diameter was measured when at a maximum just prior to its periodic expulsion. These measurements were used to calculate the amount of fluid expelled over a given period of time. All studies were made at room temperature.

EXPERIMENTAL

The contractile vacuole during feeding

Fifty individual *Tokophrya*, selected from pure cultures, which had been maintained without food for 12 to 24 hours, were mounted singly in hanging drops. Into these drops were introduced one, two or three *Tetrahymena* as food. It was found that in every case the pulsation rate increased suddenly after attachment of the prey, remained high during feeding, and fell abruptly at the termination of the feeding. On the other hand, the maximum diameter was found to vary in different individuals. Out of the 50 examined, it was larger in two, remained the same in 24 and was smaller in 24, during than before feeding. No significant differences were obtained as to pulsation rate or diameter, irrespective of whether the individuals were fed simultaneously on two, three or four *Tetrahymena*.

Table I presents the values obtained in one individual which was fed with one *Tetrahymena* and in which the maximum diameter of the contractile vacuole was less during than before feeding. The table presents in four columns (1) the intervals, in seconds, between six successive pulsations, (2) the average maximum diameters, in micra, of the contractile vacuole, (3) values calculated from the average

TABLE I

Activity of the contractile vacuole of a Tokophrya before, during and after feeding

	Intervals in seconds between six successive systoles					Av. maximum diam. of cont. vacuole	Av. amt. fluid expelled per second	Calculated number of systoles per hour
Before feeding	175''	125''	171''	185''	180''	10.4 μ	3.52 μ^3	22
During feeding	22''	23''	24''	23''	21''	9.7 μ	21.15 μ^3	159
Immediately after feeding	45''	45''	47''	45''	46''	9.7 μ	10.48 μ^3	79

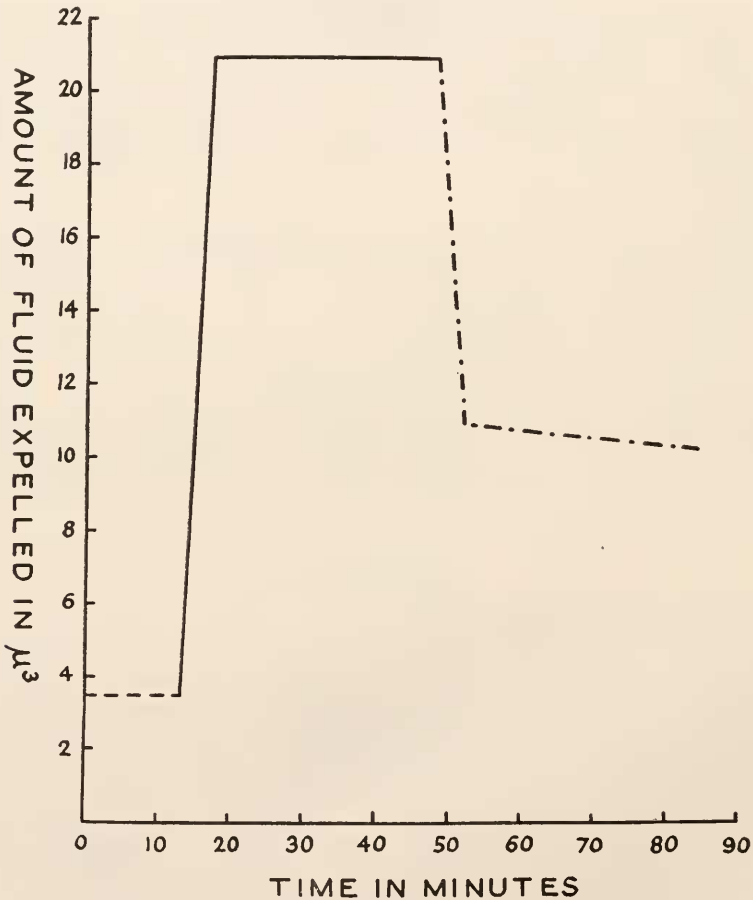


FIGURE 2. The curve represents the amount of fluid expelled by the contractile vacuole before, during and after feeding. The abscissae give the time in minutes; the ordinates, the amount of fluid in μ^3 expelled calculated for 1 sec. Data from Table I.

----- Before feeding
 _____ During feeding
 - · - · - · - After feeding

amount of fluid expelled in 1 second, and (4) the average number of systoles in one hour. From these figures it can be seen that the amount of fluid expelled by the vacuole averaged six times more during than before feeding. The amount of fluid expelled immediately after feeding was still three times as great as before feeding.

The characteristic features in these experiments are the sudden acceleration of the pulsation rate when feeding started, the increased amount of fluid expelled during and a gradual deceleration of the rate after feeding.

Figure 2 presents graphically this phenomenon. To be noted is the steepness of the upward slope representing the increase of fluid expelled when feeding has begun. The following horizontal part of the curve shows the constancy in the activity of the contractile vacuole during feeding. The subsequent rapid fall of the curve represents the decrease when feeding had ceased. Following this the curve drops gradually and, 35 minutes after feeding, is still far from the low level before feeding. Measurements were continued up to several hours after feeding at which time the level was still appreciably high.

Table II presents a striking fact that the increase in volume of the Tokophrya during feeding is appreciably less than might be expected on the basis of the volume of the food ingested. One Tetrahymena is about two to ten times larger than one

TABLE II

Volumes of three spherical Tokophrya before and after feeding. The three individuals were fed with one, two and three Tetrahymena respectively

	Fed on one Tetrahymena	Fed on two Tetrahymena	Fed on three Tetrahymena
Volume before feeding	12,000 μ^3	9,000 μ^3	11,000 μ^3
Volume after feeding	22,000 μ^3	25,000 μ^3	38,000 μ^3
Increase in volume	10,000 μ^3	16,000 μ^3	27,000 μ^3

Tokophrya. Provided the entire mass of the prey is incorporated, the Tokophrya, after having ingested one Tetrahymena, should increase in size by 200 to 1000 per cent instead of only about 80 per cent as actually occurs. This appears in the table which gives the volumes in cubic micra of each of three spherical Tokophrya before and after being fed with one, two and three Tetrahymena respectively. The table shows that in all three cases the increase in volume was definitely less than it should be when one considers the volume of the prey incorporated. The Tetrahymena is irregularly cucumber-shaped and its volume had to be estimated by considering it as an ellipsoid with long and cross axes of 60 and 24 micra respectively. The calculation gave a figure of 47,000 micra.

Calculations were also made of the volume of material actually incorporated by measuring (1) the time taken for the ingestion to take place, (2) the rate of movement of the granules through the tentacles from the prey to the host, and (3) the number, diameter and length of the predatory tentacles. The value obtained from these calculations accorded closely with that of the actual volume of the prey.

The difference between the expected and the actual increase in the volume of the host, as shown in Table II, can be accounted for by the amount of fluid expelled from the host by its contractile vacuole. This difference approximates the value

calculated from the estimated volume of the ingested *Tetrahymena* ($44,000 \mu^3$) and the amount of excess fluid expelled by the contractile vacuole of the *Tokophrya* during feeding (roughly $40,000 \mu^3$) minus the amount of fluid ($6,300 \mu^3$) expelled before feeding. The figure, $10,300 \mu^3$, which is $44,000 - (40,000 - 6,300)$, closely approximates the value of $10,000 \mu^3$, shown in Table II, of the *Tokophrya* fed with one *Tetrahymena*.

The contractile vacuole during reproduction

Experiments were performed to determine the effect of reproduction on the activity of the contractile vacuole. Unfed *Tokophrya* were used. Some were young adults kept without food since metamorphosis, others were adult *Tokophrya* which had been maintained without food for at least 12 hours.

It was found that the process of reproduction greatly accelerated the pulsation rate of the contractile vacuole and the amount of fluid expelled. This acceleration was progressive, starting from the time the brood pouch could be seen beginning to form, and reaching a maximum when the ciliated embryo was being completed as an isolated individual within the brood pouch. From that time on, the activity of the contractile vacuole of the mother decelerated, fell greatly by the time the embryo was expelled and still more so when the expulsion was complete. This last value was below the level determined at the beginning of the experiment and is to be interpreted as that of the individual before the reproductive process had started. At the time when the observations were made, the brood pouch had already begun to form and, therefore, the activity of the contractile vacuole presumably was already on the increase.

TABLE III
Activity of contractile vacuole in a Tokophrya during reproduction

Reproductive stages	Time in minutes	Intervals in seconds betw. six successive systoles					Av. maximum diam. of contr. vac. in μ	Av. amt. fluid expelled, $\mu^3/1$ sec.	Calculated number of systoles per hour
Brood pouch initiated	0	86	87	90	91	82	11.7	9.66	41
Cilia of embryo in action	15	82	80	74	67	67	11.7	11.50	49
Contractile vacuoles of embryo in action	25	44	40	37	35	35	10.4	15.50	95
Embryo being completed and starting to rotate	30	27	25	25	27	29	10.4	21.82	133
Embryo being expelled	67	55	59	60	60	60	9.8	8.36	61
Expulsion completed	75	80	90	107	120	120	9.8	4.79	35

Table III presents figures for the intervals, in seconds, between the pulsations and the maximum diameter of the contractile vacuole during successive stages of the reproductive process, *viz.*, (a) the initiation of formation of the brood pouch, (b) the beginning of the ciliary activity of the forming embryo, (c) the time when the contractile vacuoles of the embryo came into action, (d) the separation of the embryo into an isolated individual, (e) the beginning and (f) the completed expulsion of the embryo.

Figure 3 represents graphically this phenomenon. The upward slope of the curve represents the increasing vacuolar activity from the moment the brood pouch

started to be formed until the peak is reached when the embryo was fully developed. The subsequent fall represents the decrease of the vacuolar activity after the completed growth of the embryo.

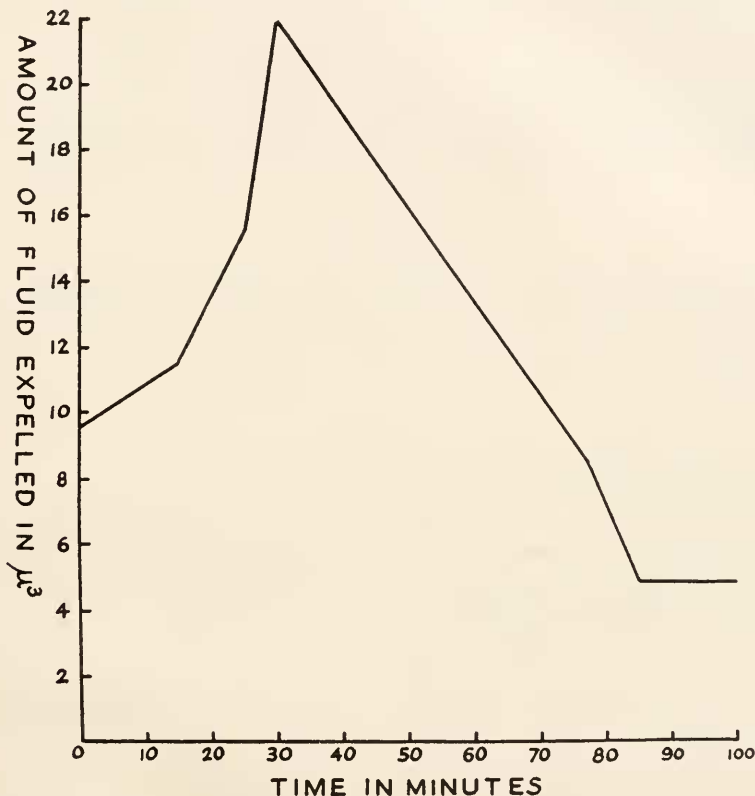


FIGURE 3. Successive changes in the amount of fluid expelled during the reproductive period. The abscissae give the time in minutes; the ordinates, the amount of fluid in μ^3 calculated for 1 sec. Data from Table III.

The contractile vacuole in individuals with abnormal reproduction in which the embryo underwent metamorphosis but persisted as an adult within the brood pouch

The contractile vacuole of these abnormal Tokophrya (Rudzinska, in press) was studied in order to ascertain whether the presence of the young adult, completely enclosed within the mother and with its tentacles attached to the wall of the brood pouch, had any effect on the activity of the contractile vacuole of the mother. In the six abnormal Tokophrya studied, it was found that the mother developed large, supernumerary contractile vacuoles which pulsated at a faster rate than normal. This is shown in Table IV, which is to be compared with Table I.

Of these abnormal individuals, four had been kept unfed. The remaining two were fed and in these the effect of feeding was evident by the still greater acceleration of the activity of the contractile vacuole.

TABLE IV

Activity of three contractile vacuoles in a Tokophrya with a young adult persisting within the brood pouch

	Intervals in seconds between six successive systoles														
	Vacuole 1					Vacuole 2					Vacuole 3				
	Before feeding	50	48	45	45	48	55	52	50	53	50	60	55	55	58
After feeding	13	13	10	10	12	10	12	10	12	12	13	10	10	12	10

DISCUSSION

In a review concerning the function of the contractile vacuole in protozoa, Weatherby (1941) refers to many investigators whose observations, he states (p. 430), "strongly suggest that the vacuole operates to prevent excessive dilution of the cytoplasm, or to regulate osmotic pressure within the cell. . . ." However, he concludes (p. 430): "In spite of the quite extensive literature dealing with the question, one is obliged to admit that virtually nothing has been proved beyond question concerning the function or functions of contractile vacuoles."

The favorable feature of *Tokophrya* is that the study of the activity of the contractile vacuole can be closely related to various phases of metabolic activity. These include feeding, reproduction and an unusual case in which the embryo underwent metamorphosis and persisted as an adult within the body of the mother.

The effect of feeding in increasing the pulsatory rate of the contractile vacuole has already been described by other investigators. Pestel (1931) observed this in the suctorian, *Dendrocometes paradoxus* Stein. Andrejewa (1931) found that in *Paramecium caudatum* the greater the number of food vacuoles, the faster is the pulsation rate of the contractile vacuole. Some investigators (Kitching, 1938) claim that the increased activity of the contractile vacuole with feeding is to be accounted for by the amount of water incorporated from the exterior during the formation of the food vacuole. However, such an explanation is excluded in *Tokophrya* since the only material incorporated is the contents of the prey. If the fluid expelled is only that taken in unchanged from the Tetrahymena it would be expected that when feeding is completed the pulsation rate should return soon to the previous rate. However, this does not occur. The contraction rate falls only gradually and still is appreciably high for as long as several hours after feeding. This indicates the possibility of another factor responsible for the persisting high rate of output, namely, an increase in the metabolic activity of the *Tokophrya*. Such a relation is supported by the findings of Shumway (1917), Riddle and Torrey (1922) and Flather (1919) that thyroxin, adrenalin, pituitary and pineal gland extract accelerate the pulsation rate of the contractile vacuole. The correlation between the activity of the contractile vacuole and increased metabolism is also suggested in those cases of *Tokophrya* in which the young adult persisted virtually as a parasite within the brood pouch of the mother. The increased number and rapid contraction rates of the contractile vacuoles in these mothers may be accounted for by the metabolic activity of the imprisoned, parasitic offspring. The contractile vacuoles of the mother evidently are doing double duty for herself and for her enclosed adult offspring.

The increased rate of pulsation during reproduction has also been described in the literature. Unger (1925, 1926) studied the activity of the contractile vacuole of *Paramecium caudatum*, *P. aurelia* and *P. calkinsi* in connection with their division rate and during endomixis. He found a relation between a reduction in the pulsatile rate of the contractile vacuole and a reduction in the rate of division and of endomixis. Dimitrowa (1928) observed that at the beginning of mitosis in *Paramecium caudatum* two additional contractile vacuoles appear and claimed this to indicate increased excretory activity. Pestel (1931) observed that the first sign for endogenous budding in *Dendrocometes paradoxus*, a suctorian, is the formation of a small contractile vacuole in that part of the cytoplasm which is destined to be the embryo.

A feature of this paper is that it has been possible to show in the same species, *Tokophrya*, three kinds of functional activity to each of which the contractile vacuole responds specifically. These are a response (1) to feeding, (2) to the process of reproduction, and (3) to the parasitic existence of the offspring enclosed within the body of the host.

SUMMARY

1. Evidence is presented for the existence of a close relation between the behavior of the contractile vacuole and several phases of the functional activity of *Tokophrya infusionum*.

2. When the process of feeding starts the pulsation rate of the contractile vacuole rises suddenly, remains high during feeding and falls gradually on cessation of the feeding process. It is significant that the pulsation rate remains comparatively high for several hours after the termination of the feeding.

3. During reproduction the pulsation rate of the contractile vacuole increases as soon as the endogenous budding starts, rises and remains high until the ciliated embryo has become separated within the brood pouch. This moment is the high peak of the vacuolar activity. The pulsation rate subsequently begins to decrease and falls greatly upon expulsion of the embryo. After expulsion, which lasts several minutes, the pulsation rate of the contractile vacuole drops still lower.

4. Unusually accelerated activity of the contractile vacuole was noted in those cases in which metamorphosis of the embryo occurred within the parent and the resulting young adult persisted with the tips of its tentacles attached to the wall of brood pouch.

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